

Fusion primer and nested integrated PCR (FPNI-PCR)

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 An abbreviated version of this protocol was published in BMC Plant Biology in Mar 2021

Genetic analysis of the barley variegation mutant, grandpa1.a

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Detailed protocol

Protocol for Tail-PCR/ FPNI-PCR:

Round1:

10x LA buffer – 1ul

MgCl₂ – 1ul

dNTP (2.5mM) – 1.6ul

F primers – 4ul

(Separate PCR for each of the FP primers 1-9)

R primer (gene-specific) – 0.8ul

DNA (100ng/ul) – 1.5ul

LA Taq – 0.1ul

H₂O – 0ul

Total Volume=10ul

Round1 with DMSO: (This method gave better results. Gpa1 has a high GC content)

10x LA buffer – 1ul

MgCl₂ – 1ul

dNTP (2.5mM) – 1.6ul

F primers – 4ul

(separate PCR for each of the FAD primers 1-9.)

R primer (gene-specific) – 0.8ul

DNA (100ng/ul) – 1.3ul

DMSO – 0.2ul

LA Taq – 0.1ul

H₂O – 0ul

Total Volume=10ul

Round1 Cycle:

1. 95 °C - 1:30
2. 94 °C - 0:10
3. 62 °C – 0:30
4. 72 °C - 2:00
5. 94 °C – 0:15
6. 25 °C – 1:00
7. 28 °C, increase 0.2°C/sec – 3:00
8. 72 °C – 2:30
9. Go To Step 2 5x (total of 6x)
10. 94 °C – 0:10
11. 62 °C – 0:30
12. 72 °C C – 2:00
13. Go To Step 10 1x (total 2x)
14. 94 °C – 0:10
15. 44 °C – 0:30
16. 72 °C – 2:00

17. 72 °C – 5:00
18. 10 °C - forever

Round 2:

Dilute Round1 PCR product ½ to use as template. Still keep the FP samples 1-9 separate.
(Note: Most genes will need 5x HF buffer and no DMSO. *Gpa1* contains high-GC regions though)

5x GC Rich Phusion Buffer – 4ul
dNTP (2.5mM) – 1.6ul
FSP1 – 4ul
Gene specific R (nested) – 1.6ul
DMSO – 0.6
½ conc. PCR template – 1ul
Phusion – 0.2ul
H₂O – 4.6ul

Cycle conditions:

1. 98 °C – 1:30
2. 98 °C – 0:10
3. 61 °C – 0:30
4. 72 °C – 1:30
5. Go To step 2 34x (total of 35x)
6. 72 °C – 5:00
7. 10 °C – forever

Round 3:

Dilute Round2 PCR product 1/40 to use as template. Still keep the FP samples 1-9 separate.
(Note: Most genes will need 5x HF buffer and no DMSO. *Gpa1* was a high-GC content region though)

5x GC Rich Phusion Buffer – 4ul
dNTP (2.5mM) – 1.6ul
FSP2 – 4ul
Gene specific R (nested) – 1.6ul
DMSO – 0.6
1/40 conc. PCR template – 1ul
Phusion – 0.2ul
H₂O – 4.6ul

Cycle conditions:

1. 98 °C – 1:30
2. 98 °C – 0:10
3. 64 °C – 0:30
4. 72 °C – 1:30
5. Go To step 2 34x (total of 35x)
6. 72 °C – 5:00
7. 10 °C – forever

Final steps:

Run all 9 samples on 1% agarose gel. Use a gel extraction kit to excise and purify the major bands. Send for sequencing.

Primers:

Will need 3 gene specific primers, each nesting closer to the unknown sequence region.
9 different FP primers, and 2 “nesting” FSP primers:

FP1	GTAATACGACTCACTATAGGGCACGCGTGGTNTCGASTWTSGWGTT
FP2	GTAATACGACTCACTATAGGGCACGCGTGGTNGTCGASWGANAWGAA
FP3	GTAATACGACTCACTATAGGGCACGCGTGGTWGTGNAGWANCANAGA
FP4	GTAATACGACTCACTATAGGGCACGCGTGGTAGWGNAGWANCAWAGG
FP5	GTAATACGACTCACTATAGGGCACGCGTGGTNGTAWAASGNTNTSCAA
FP6	GTAATACGACTCACTATAGGGCACGCGTGGTNGACGASWGANAWGAC
FP7	GTAATACGACTCACTATAGGGCACGCGTGGTNGACGASWGANAWGAA
FP8	GTAATACGACTCACTATAGGGCACGCGTGGTGTNCGASWCANAWGTT

FP9	GTAATACGACTCACTATAGGGCACGCGTGGTNCAGCTWSCTNTSCTT
FSP1	GTAATACGACTCACTATAGGGC
FSP2	ACTATAGGGCACGCGTGGT

Acronyms:

FP = "Fusion arbitrary degenerate primers", also called FAD primers.

FSP = "FP-specific primers"

Please refer to Wang et al. (2011).

Reference:

Wang Z, Ye S, Li J, Zheng B, Bao M, Ning G. Fusion primer and nested integrated PCR (FPNI-PCR): a new high-efficiency strategy for rapid chromosome walking or flanking sequence cloning. BMC Biotechnol. 2011;11:109.

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1. Yang, S. (2022). Fusion primer and nested integrated PCR (FPNI-PCR). Bio-protocol Preprint. bio-protocol.org/preprint1810.
2. Yang, S., Overlander, M. and Fiedler, J.(2021). Genetic analysis of the barley variegation mutant, grandpa1.a. BMC Plant Biology 0(0). DOI: [10.1186/s12870-021-02915-9](https://doi.org/10.1186/s12870-021-02915-9)

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